Claims

1. A method for the preparation of a polypeptide of interest in authentic form, said method comprising the steps of:
(i) providing a fusion protein comprising, from its N-terminal to its C-terminal, (a) a fusion partner, (b) a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site, (c) a polypeptide of interest, wherein said cleavage site being adjacent to the polypeptide of interest, and
(ii) contacting said fusion protein with Granzyme B protease to cleave it at said cleavage site to yield said polypeptide of interest in authentic form.

2. A method according to claim 1, wherein the Granzyme B protease recognition site has an amino acid sequence of the general formula:

P4 P3 P2 P1↓

wherein

P4 is amino acid I or V

P3 is amino acid E, Q or M

P2 is X, where X denotes any amino acid,

P1 is amino acid D, and

↓ is the cleavage site for said Granzyme B protease.

3. A method according to claim 1, wherein the Granzyme B protease recognition site has an amino acid sequence selected from the group consisting of ICPD↓, IEAD↓, IEPD↓, IETD↓, IQAD↓, ISAD↓, ISSD↓, ITPD↓, VAPD↓, VATD↓, VCTD↓, VDPD↓, VDSD↓, VEKD↓, VEQD↓, VGPD↓, VEID↓, VRPD↓, VTPD↓, LEED↓, LEID↓, LGND↓, LGPD↓, AQPD↓, and wherein ↓ is the cleavage site for said Granzyme B protease.

4. A method according to claim 2, wherein the general formula furthermore comprises the amino acids P1' and P2' resulting in the general formula
P4 P3 P2 P1↓P1'P2', wherein P1' is X where X denotes any amino acid, P2' is G, and wherein P1' and P2' is a part of the polypeptide of interest.

5. A method according to claim 2, wherein the general formula furthermore comprises the amino acids P1', P2', P3' and P4' resulting in the general formula P4 P3 P2 P1↓P1'P2'P3'P4', wherein P4' is D or E, and wherein P1', P2', P3' and P4' is a part of the polypeptide of interest.

6. A method according to claim 1, wherein the polypeptide of interest is selected from the group consisting of an enzyme, a polypeptide hormone, a single chain antibody variable region fragment, and apolipoprotein A.

7. A method according to claim 6, wherein the polypeptide hormone is selected from the group consisting of somatotrophin, glucagon, insulin and inteferon.

8. A method according to claim 6, wherein the enzyme is Granzyme B.

9. A method according to claim 1, wherein the fusion partner is an affinity-tag.

10. A method according to claim 9, wherein the affinity-tag is selected from the group consisting of a polyhistidine-tag, a polyarginine-tag, a FLAG-tag, a Strep-tag, a c-myc-tag, a S-tag, a calmodulin-binding peptide, a cellulose-binding peptide, a chitin-binding domain, a glutathione S-transferase-tag, and a maltose binding protein.

11. A method according to claim 1, wherein the Granzyme B protease is selected from the group consisting of human Granzyme B protease, mouse Granzyme B protease and rat Granzyme B protease.

12. A method according to claim 11, wherein the Granzyme B protease is a human Granzyme B protease variant as shown in SEQ ID NO 57, wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.

13. A method according to claim 1, wherein the Granzyme B protease is in an immobilised form.

14. A method according to claim 13, wherein the Granzyme B protease is immobilised via the C-terminus.

15. A method according to claim 13, wherein the Granzyme B protease is immobilised via a lysine amino acid residue.

16. A method according to claim 10, wherein the affinity-tag is a polyhistidine-tag, and wherein the fusion protein is contacted with Granzyme B protease in the presence of Ni^{2+} ions and Nitrilotriacetic Acid (NTA).

17. A method according to claim 15, wherein the concentration of Ni^{2+} is in the range of 1-20 mM, and the concentration of NTA is in the range of 1-20 mM.

18. A fusion protein comprising, from its N-terminal to its C-terminal, (a) a fusion partner, (b) a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site, and (c) a polypeptide of interest, wherein said cleavage site being adjacent to the polypeptide of interest.

19. A fusion protein according to claim 18, wherein the Granzyme B protease recognition site has an amino acid sequence of the general formula:

P4 P3 P2 P1↓

wherein

P4 is amino acid I or V

P3 is amino acid E, Q or M

P2 is X, where X denotes any amino acid,

P1 is amino acid D, and

↓ is the cleavage site for said Granzyme B protease.

20. A fusion protein according to claim 18, wherein the Granzyme B protease recognition site has an amino acid sequence selected from the group consisting of ICPD↓, IEAD↓, IEPD↓, IETD↓, IQAD↓, ISAD↓, ISSD↓, ITPD↓, VAPD↓, VATD↓, VCTD↓, VDPD↓, VDSD↓, VEKD↓, VEQD↓, VGPD↓, VEID↓, VRPD↓, VTPD↓, LEED↓,

LEID↓, LGND↓, LGPD↓, AQPD↓, and wherein ↓ is the cleavage site for said Granzyme B protease.

21. A fusion protein according to claim 19, wherein the general formula furthermore comprises the amino acids P1' and P2' resulting in the general formula P4 P3 P2 P1↓P1'P2', wherein P1' is X where X denotes any amino acid, P2' is G, and wherein P1' and P2' is a part of the polypeptide of interest.

22. A fusion protein according to claim 19, wherein the general formula furthermore comprises the amino acids P1', P2', P3' and P4' resulting in the general formula P4 P3 P2 P1↓P1'P2'P3'P4', wherein P4' is D or E, and wherein P1', P2', P3' and P4' is a part of the polypeptide of interest.

23. A fusion protein according to claim 18, wherein the polypeptide of interest is selected from the group consisting of an enzyme, a polypeptide hormone, a single chain antibody variable region fragment, and apolipoprotein A.

24. A fusion protein according to claim 23, wherein the polypeptide hormone is selected from the group consisting of somatotrophin, glucagon, insulin and inteferon.

25. A fusion protein according to claim 23, wherein the enzyme is Granzyme B.

26. A fusion protein according to claim 25, wherein Granzyme B comprises a C-terminal polyhistidine-tag.

27. A fusion protein according to claim 25, selected from the group consisting of pro-IEPD-GrB-H6 (SEQ ID NO 2) and pro-IEAD-GrB-H6 (SEQ ID NO 3).

28. A fusion protein according to claim 25, selected from the group consisting of pro-IEPD-GrB-H6 C228A (SEQ ID NO 5), pro-IEPD-GrB-H6 C228T (SEQ ID NO 6), pro-IEPD-GrB-H6 C228V (SEQ ID NO 7), and pro-IEPD-GrB-H6 C228F (SEQ ID NO 8).

29. A fusion protein according to claim 25, wherein the enzyme Granzyme B is a human Granzyme B protease variant wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.

30. A fusion protein according to claim 25, wherein the human Granzyme B protease variant is as shown in SEQ ID NO 57.

31. A fusion protein according to claim 18, wherein the fusion partner is an affinity-tag.

32. A fusion protein according to claim 31, wherein the affinity-tag is selected from the group consisting of a polyhistidine-tag, a polyarginine-tag, a FLAG-tag, a Strep-tag, a c-myc-tag, a S-tag, a calmodulin-binding peptide, a cellulose-binding peptide, a chitin-binding domain, a glutathione S-transferase-tag, and a maltose binding protein.

33. A human Granzyme B protease variant wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.

34. A human Granzyme B protease variant according to claim 33, as shown in SEQ ID NO 57.

35. Use of a human Granzyme B protease variant according to claim 33 or 34.

36. An isolated nucleic acid sequence encoding the fusion protein according to any of claims 19-32 or the human Granzyme B protease variant according to any of claims 33 or 34.

37. A recombinant vector comprising the isolated nucleic acid sequence according to claim 36.

38. A host cell transformed with a vector according to claim 37.

39. A method for the production of a fusion protein according to claim 18 or a human Granzyme B protease variant according to claim 33 or 34, comprising the steps of:

(i) providing a recombinant vector comprising the isolated nucleic acid sequence according to claim 36 operatively linked to a promotor,

(ii) transforming a host cell with said recombinant vector,

(iii) culturing said host cell under conditions to express said fusion protein or human Granzyme B protease variant, and

(iv) optionally isolating said fusion protein or human Granzyme B protease variant.